SEP 29 2004
PATENT AND TRADEMARK OFFICE

DOCKET NO.: M0925.70114US01

Exhibit III

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Katrin Kneipp, et al.
Serial No.:	10/723,174
Confirmation No.:	5755
Filed:	November 26, 2003
For:	SINGLE MOLECULE DETECTION WITH SURFACE-ENHANCED RAMAN SCATTERING AND APPLICATIONS IN DNA OR RNA SEQUENCING
Examiner:	Not Yet Assigned
Art Unit:	1645

MAIL STOP Amendment
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith are the following documents:

- Preliminary Amendment
- Return Receipt Postcard

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 646-8000, Boston, Massachusetts.

A check is not enclosed. If a fee is required, the Commissioner is hereby authorized to charge Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

Respectfully submitted,

By: _____

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Date: September 29, 2004
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Express Mail Certificate Number: EV 292564108 US
Date of Deposit: September 29, 2004

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PRELIMINARY AMENDMENT

Sir:

Before beginning examination of the above-identified application on its merits, please amend the application as follows:

The Claims are reflected in the listing of claims that begins on page 2.

Remarks begin on page 24.

Express Mail Certificate Number: EV 292564108 US
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In the Claims

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1. (Original) A method for determining the presence of at least one analyte, comprising:

providing a sample comprising a plurality of aggregates of size of at least about 500 nm adsorbing a plurality of analytes;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein at least one spectral line of the information represents a single analyte adsorbed on one of the plurality of aggregates; and

determining the presence of the single analyte from the at least one spectral line.

2. (Original) A method as in claim 1, the exposing step involving exposing the sample to electromagnetic radiation and causing Raman scattering of the sample, and the obtaining step comprising obtaining Raman information of the sample, wherein a single Raman line of the information represents the single analyte.

3. (Original) A method as in claim 1, wherein the sample is free of an emission-enhancing aid.

4. (Original) A method as in claim 1, wherein the spectral information is a surface-enhanced Raman spectrum, having an enhancement factor of at least about 10^{10}.

5. (Original) A method as in claim 1, wherein each aggregate of the plurality of aggregates comprises a plurality of metal particles.

6. (Original) A method as in claim 5, wherein the plurality of metal particles is selected from the group consisting of silver, gold and copper particles.

7. (Original) A method as in claim 6, wherein the aggregate is formed in situ by exposure to the electromagnetic radiation.

8. (Original) A method as in claim 1, wherein the plurality of aggregates is selected from the group consisting of colloids suspended in a medium, aggregates deposited on a substrate and lithography-produced metal aggregates.

9. (Original) A method as in claim 8, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

10. (Original) A method as in claim 8, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

11. (Original) A method as in claim 1, wherein the sample consists essentially of a plurality of aggregates of from about 500 nm to about 20 microns in dimension.

12. (Original) A method as in claim 1, wherein the electromagnetic radiation is non-resonant radiation.

13. (Original) A method as in claim 12, wherein the electromagnetic radiation is near infrared radiation.

14. (Original) A method as in claim 1, wherein the spectral information is Raman information that defines less than a complete Raman spectrum.

15. (Original) A method as in claim 14, wherein the spectral information is less than 5 Raman lines.

16. (Original) A method as in claim 14, wherein the spectral information is less than 2 Raman lines.

17. (Original) A method as in claim 1, wherein the spectral information is a single Raman line.

18. (Original) A method as in claim 1, wherein the single analyte is a dye.

19. (Original) A method as in claim 1, wherein the single analyte is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

20. (Original) A method as in claim 1, wherein the single analyte is selected from the group consisting of nucleotides and nucleosides.

21. (Original) A method as in claim 1, wherein the single analyte is a therapeutic agent.

22. (Original) A method as in claim 1, wherein the single analyte is a neurotransmitter.

23. (Original) A method for determining the presence of an analyte, comprising:

providing a sample comprising a plurality of aggregates adsorbing a plurality of analytes, wherein at least one aggregate of the plurality of aggregates comprises a metal cluster of at least seven particles and adsorbs only one analyte;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein the only one analyte contributes to the spectral information; and

determining the presence of the only one analyte from the spectral information.

24. (Original) A method as in claim 23, the exposing step involving exposing the sample to electromagnetic radiation to cause Raman scattering, and the obtaining step involves obtaining a Raman spectrum of the sample, wherein the only one analyte contributes to at least one Raman signal of the Raman spectrum.

25. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least ten particles.

26. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least twenty particles.

27. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least thirty-five particles.

28. (Original) A method as in claim 23, wherein the sample is free of an emission-enhancing aid.

29. (Original) A method as in claim 23, wherein the Raman spectrum is a surface-enhanced Raman spectrum, having an enhancement factor of at least 1010.

30. (Original) A method as in claim 23, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

31. (Original) A method as in claim 23, wherein the aggregate is formed in situ by exposure to the electromagnetic radiation.

32. (Original) A method as in claim 23, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

33. (Original) A method as in claim 32, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

34. (Original) A method as in claim 32, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

35. (Original) A method as in claim 23, wherein the at least one aggregate has a dimension of at least about 500 nm.

36. (Original) A method as in claim 23, wherein the electromagnetic radiation is non-resonant radiation.

37. (Original) A method as in claim 36, wherein the electromagnetic radiation is near infrared radiation.

38. (Original) A method as in claim 23, wherein the single analyte is a dye.

39. (Original) A method as in claim 23, wherein the single analyte is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

40. (Original) A method as in claim 23, wherein the single analyte is selected from the group consisting of nucleotides and nucleosides.

41. (Original) A method as in claim 23, wherein the single analyte is a therapeutic agent.

42. (Original) A method as in claim 23, wherein the single analyte is a neurotransmitter.

43. (Original) A method as in claim 23, wherein the sample consists essentially of aggregates of size of from about 500 nm to about 20 microns.

44. (Original) A method as in claim 23, wherein the at least one aggregate comprises a plurality of metal particles each having a dimension of no more than about 100 nm.

45. (Original) A method as in claim 23, wherein the at least one aggregate comprises a plurality of metal particles each having a dimension of no more than about 75 nm.

46. (Original) A method for determining the presence of an analyte, comprising:

providing a sample comprising a plurality of aggregates adsorbing a plurality of analytes, wherein each aggregate comprises a plurality of metal particles, each metal particle having a dimension of no more than about 100 nm and at least one aggregate adsorbs only one analyte;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein the only one analyte contributes to the spectral information; and

determining the presence of the only one analyte from the spectral information.

47. (Original) A method as in claim 46, wherein the exposing step involves causing surface-enhanced emission and the obtaining step involves obtaining Raman spectral information.

48. (Original) A method as in claim 46, wherein the sample is free of an emission-enhancing aid.

49. (Original) A method as in claim 46, wherein the spectral information is a surface-enhanced Raman spectrum, having an enhancement factor of at least 1010.

50. (Original) A method as in claim 46, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

51. (Original) A method as in claim 46, wherein the aggregate is formed in situ by exposure to the electromagnetic radiation.

52. (Original) A method as in claim 46, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

53. (Original) A method as in claim 52, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

54. (Original) A method as in claim 52, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

55. (Original) A method as in claim 46, each metal particle having a dimension of no more than about 75 nm.

56. (Original) A method as in claim 46, wherein the electromagnetic radiation is non-resonant radiation.

57. (Original) A method as in claim 56, wherein the electromagnetic radiation is near infrared radiation.

58. (Original) A method as in claim 46, wherein the spectral information consists essentially of less than 5 lines of a Raman spectrum.

59. (Original) A method as in claim 46, wherein the single analyte is a dye.

60. (Original) A method as in claim 46, wherein the single analyte is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

61. (Original) A method as in claim 46, wherein the single analyte is selected from the group consisting of nucleotides and nucleosides.

62. (Original) A method as in claim 46, wherein the single analyte is a therapeutic agent.

63. (Original) A method as in claim 46, wherein the single analyte is a neurotransmitter.

64. (Original) A method for determining the presence of at least one analyte, comprising:

providing a sample comprising a plurality of aggregates, at least one aggregate adsorbing only one analyte that is free of an emission-enhancing aid;

exposing the sample to electromagnetic radiation; and

obtaining a spectrum, wherein the only one analyte contributes to at least one signal of the spectrum.

65. (Original) A method as in claim 64, wherein the spectrum is a surface-enhanced Raman spectrum, having an enhancement factor of at least 1010.

66. (Original) A method as in claim 64, wherein each aggregate of the plurality of aggregates comprises a plurality of metal particles.

67. (Original) A method as in claim 66, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

68. (Original) A method as in claim 64, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

69. (Original) A method as in claim 64, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

70. (Original) A method as in claim 69, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

71. (Original) A method as in claim 69, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

72. (Original) A method as in claim 64, wherein the at least one aggregate has a dimension of at least about 500 nm.

73. (Original) A method as in claim 64, wherein the single analyte is a dye.

74. (Original) A method as in claim 64, wherein the single analyte is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

75. (Original) A method as in claim 64, wherein the single analyte is selected from the group consisting of nucleotides and nucleosides.

76. (Original) A method as in claim 64, wherein the single analyte is a therapeutic agent.

77. (Original) A method as in claim 64, wherein the single analyte is a neurotransmitter.

78. (Original) A method for determining the presence of a single analyte, comprising:

providing a sample comprising a plurality of surfaces, a portion of the plurality of surfaces adsorbing only one analyte; and

exposing the sample to electromagnetic radiation to cause the sample to emit radiation such that the sample is free of photobleaching.

79. (Original) A method as in claim 78, wherein the plurality of surfaces comprises a plurality of aggregates.

80. (Original) A method as in claim 79, wherein the plurality of aggregates comprises a plurality of metal particles.

81. (Original) A method as in claim 80, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

82. (Original) A method as in claim 79, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

83. (Original) A method as in claim 82, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

84. (Original) A method as in claim 82, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

85. (Original) A method as in claim 78, wherein the plurality of surfaces comprises a plurality of aggregates of metal particles, each of the metal particles having a dimension of no more than about 100 nm.

86. (Original) A method as in claim 78, wherein the only one analyte is a dye.

87. (Original) A method as in claim 78, wherein the only one analyte is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

88. (Original) A method as in claim 78, wherein the only one analyte is selected from the group consisting of nucleotides and nucleosides.

89. (Original) A method as in claim 78, wherein the only one analyte is a therapeutic agent.

90. (Original) A method as in claim 78, wherein the only one analyte is a neurotransmitter.

91. (Original) A method for determining the presence of at least one molecule, comprising providing at least one molecule, exposing the at least one molecule to electromagnetic radiation to cause Raman scattering, obtaining Raman spectral information and determining the presence of the at least one molecule from at least one anti-Stokes line.

92. (Original) A method as in claim 91, wherein the at least one molecule is adsorbed on a plurality of surfaces.

93. (Original) A method as in claim 91, wherein the at least one analyte is exposed to non-resonant radiation.

94. (Original) A method as in claim 92, wherein the electromagnetic radiation is near infrared radiation.

95. (Original) A method as in claim 94, wherein the near infrared radiation has a wavelength of at least 1000 nm.

96. (Original) A method for sequencing at least a portion of DNA or RNA, comprising:

cleaving the at least a portion of DNA or RNA into DNA or RNA fragments, wherein each fragment comprises at least one base;

allowing each DNA or RNA fragment to become surface-adsorbed;

exposing each fragment to electromagnetic radiation to cause surface-enhanced emission; and

obtaining unique surface-enhanced spectral information attributed to each fragment.

97. (Original) A method as in claim 96, wherein each fragment is surface-adsorbed onto one of a plurality of surfaces.

98. (Original) A method as in claim 97, wherein the plurality of surfaces is included in a moving stream.

99. (Original) A method as in claim 97, wherein the plurality of surfaces is selected from the group consisting of a plurality of aggregates suspended in a medium, a plurality of aggregates deposited on a substrate and lithography produced metal aggregates.

100. (Original) A method as in claim 99, wherein the plurality of aggregates comprise clusters of metal particles.

101. (Original) A method as in claim 100, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

102. (Original) A method as in claim 99, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

103. (Original) A method as in claim 100, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

104. (Original) A method as in claim 96, comprising allowing each fragment to become surface-absorbed on a plurality of protrusions and voids on a rough metal film.

105. (Original) A method as in claim 96, wherein the electromagnetic radiation is non-resonant radiation.

106. (Original) A method as in claim 96, wherein the electromagnetic radiation is near infrared radiation.

107. (Original) A method for general field enhancement, comprising providing a plurality of aggregates, exposing the plurality of aggregates to near infrared radiation and inducing at least one electromagnetic resonance in the plurality of aggregates to cause a surface-enhanced radiation.

108. (Original) A method as in claim 107, wherein the near infrared radiation has a wavelength of at least 1000 nm.

109. (Original) A method as in claim 107, wherein the plurality of aggregates comprises a plurality of metal particles.

110. (Original) A method as in claim 109, wherein the metal particles are selected from the group consisting of silver, gold and copper particles.

111. (Original) A method as in claim 107, wherein the aggregate is formed in situ by exposure to the electromagnetic radiation.

112. (Original) A method as in claim 107, wherein the plurality of aggregates is selected from the group consisting of colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

113. (Original) A method as in claim 112, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

114. (Original) A method as in claim 112, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

115. (Original) A method as in claim 109, wherein each metal particle has a dimension of no more than about 100 nm.

116. (Original) A method as in claim 109, wherein the plurality of aggregates comprises at least seven metal particles.

117. (Original) A method as in claim 107, wherein the surface enhanced radiation has an enhancement factor of at least 1010.

118. (Original) A method for selecting a spectral range, comprising:

providing a sample;

positioning at least one filter in association with an optical excitation and detection system, wherein the system is free of a spectrograph and the optical excitation system produces electromagnetic radiation in a first range;

exposing the sample to electromagnetic radiation via the system; and

obtaining a Raman spectrum of the sample having a second range wherein the second range is shifted from the first range.

119. (Original) A method as in claim 118, involving positioning at least two filters in association with the optical excitation and detection system.

120. (Original) A method as in claim 118, the positioning step involving positioning the at least one filter between a sample and detector of a Raman spectral system.

121. (Original) A method as in claim 118, wherein the second range is narrower than the first range.

122. (Original) A method for determining the presence of an analyte, comprising:

providing a sample comprising a rough metal film including a plurality of protrusions and indentations;

absorbing a plurality of analytes on a surface of the film;

exposing the sample to electromagnetic radiation to cause Raman scattering; and

obtaining a unique Raman signal attributed to a single analyte.

123. (Original) A system for determining the presence of at least one analyte, comprising:

a sample;

a source of electromagnetic radiation positioned to irradiate the sample; and

a detector positioned to detect surface-enhanced emission from the sample,

wherein the sample comprises a plurality of aggregates of size of at least about 500 nm.

124. (Original) A system as in claim 123, wherein the sample comprises a plurality of aggregates of size of at least about 500 nm on a substrate.

125. (New) A method comprising:

a) sequentially removing nucleotides from one end of at least one nucleic acid;

b) attaching each nucleotide to at least one nanoparticle;

c) identifying said nucleotides; and

d) determining the sequence of said nucleic acid.

126. (New) The method of claim 125, wherein said nucleic acid is attached to a surface.

127. (New) The method of claim 125, wherein said nanoparticles comprise a modified surface.

128. (New) The method of claim 125, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

129. (New) The method of claim 125, wherein said nanoparticles comprise gold and/or silver.

130. (New) The method of claim 129, wherein each nucleotide is attached to a single nanoparticle or a nanoparticle aggregate.

131. (New) The method of claim 125, further comprising separating said nucleotides from said nucleic acid molecule.

132. (New) The method of claim 128, further comprising exciting said nucleotides with a laser.

133. (New) The method of claim 132, wherein a charge coupled device (CCD) camera is used to identify said nucleotides.

134. (New) The method of claim 125, further comprising recording the identity of each nucleotide and the time at which each nucleotide is identified.

135. (New) The method of claim 125, wherein an exonuclease is used to remove said nucleotides from said nucleic acid.

136. (New) The method of claim 125, wherein said nanoparticles are between 10 nm and 20 micrometers in diameter.

137. (New) The method of claim 136, wherein said nanoparticles are about 100 nm in diameter.

138. (New) A method comprising:

a) obtaining nucleotides that are attached to Raman labels;

b) providing a nucleic acid comprising labeled nucleotides;

c) removing nucleotides from one end of the nucleic acid;

d) identifying nucleotides by Raman spectroscopy; and

e) determining the sequence of the nucleic acid.

139. (New) The method of claim 138, further comprising passing the nucleotides removed from the nucleic acid in a stream.

140. (New) The method of claim 138, wherein each type of nucleotide is labeled with a Raman label.

141. (New) The method of claim 138, comprising labeling thymine.

142. (New) The method of claim 138, comprising labeling adenine.

143. (New) The method of claim 138, comprising labeling cytosine.

144. (New) The method of claim 138, comprising labeling guanine.

145. (New) The method of claim 138, comprising labeling uracil.

146. (New) The method of claim 138, wherein said nucleotides are removed from said nucleic acid by exonuclease activity.

147. (New) The method of claim 146, wherein only one nucleic acid at a time is exposed to exonuclease activity.

148. (New) The method of claim 138, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

149. (New) The method of claim 148, further comprising attaching said nucleotides to nanoparticles.

150. (New) An apparatus comprising:

a) a reaction site for immobilizing a DNA fragment onto an aggregate;

b) a first channel carrying a liquid stream, the first channel in fluid communication with said reaction site;

c) a second channel carrying the liquid stream, the second channel in fluid communication with said first channel;

d) a detection site in fluid communication with said first and second channels; and

e) a detection unit operably coupled to said detection site.

151. (New) The apparatus of claim 150, wherein said detection unit comprises a Raman detector.

152. (New) The apparatus of claim 151, wherein said detection unit comprises a laser and a CCD camera.

153. (New) A method comprising:

a) sequentially removing nucleotides from one end of at least one nucleic acid;

b) moving the nucleotides in a stream packed with nanoparticles;

c) identifying the nucleotides by Raman spectroscopy; and

d) determining the sequence of the nucleic acid.

154. (New) The method of claim 153, wherein the nucleotides are removed from the nucleic acid by exonuclease activity.

155. (New) The method of claim 153, further comprising attaching said nucleic acid to a surface.

156. (New) The method of claim 155, wherein said nucleic acid is immobilized in a reaction site.

157. (New) The method of claim 156, wherein a single nucleic acid is immobilized in said reaction site.

158. (New) The method of claim 153, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

159. (New) The method of claim 153, wherein at least two nanoparticles are cross-linked together.

160. (New) The method of claim 153, wherein the nanoparticles comprise gold and/or silver, said nanoparticles between about 10 nm and 20 micrometers in size.

161. (New) The method of claim 160, wherein the size of said nanoparticles is selected from the group consisting of about 10 to 50 nm, about 10 to 100 nm, about 10 nm and about 500 nm.

162. (New) A method comprising:

a) preparing a nucleic acid comprising labeled nucleotides;

b) sequentially removing nucleotides from one end of the nucleic acid;

c) moving the nucleotides in a stream packed with nanoparticles;

d) identifying the nucleotides by Raman spectroscopy; and

e) determining the sequence of the nucleic acid.

163. (New) The method of claim 162, wherein said nucleotides are labeled with one or more Raman labels.

164. (New) The method of claim 163, wherein each type of nucleotide is labeled with a distinguishable Raman label.

165. (New) The method of claim 163, comprising labeling thymine.

166. (New) The method of claim 163, comprising labeling adenine.

167. (New) The method of claim 163, comprising labeling cytosine.

168. (New) The method of claim 163, comprising labeling guanine.

169. (New) The method of claim 163, comprising labeling uracil.

170. (New) The method of claim 162, further comprising separating said nucleotides from said nucleic acid.

171. (New) The method of claim 162, further comprising recording the time at which each nucleotide passes through said channel.

172. (New) The method of claim 13, wherein each type of nucleotide produces a unique Raman signal.

173. (New) An apparatus comprising:

a) a reaction site for immobilizing a DNA fragment onto an aggregate;

b) a first channel carrying a liquid stream, the first channel in fluid communication with said reaction site;

c) a second channel carrying the liquid stream, the second channel in fluid communication with said first channel;

d) a multiplicity of nanoparticles in said second channel; and

e) a Raman detector operably coupled to said second channel.

174. (New) The apparatus of claim 173, wherein said Raman detector comprises a laser and/or a CCD camera.

175. (New) The apparatus of claim 173, further comprising a first electrode and a second electrode, said electrodes to move nucleotides from said first channel into said second channel.

176. (New) The apparatus of claim 173, wherein said nanoparticles are cross-linked together.

177. (New) The apparatus of claim 176, wherein said cross-linked nanoparticles provide an enhanced Raman signal.

178. (New) The apparatus of claim 173, said detector to detect nucleotides by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

179. (New) A method comprising:

a) removing one or more nucleotides from a nucleic acid;

b) attaching each of the one or more nucleotides to at least one nanoparticle;

c) identifying said nucleotides; and

d) determining the sequence of said nucleic acid.

180. (New) The method of claim 179, wherein said nanoparticles comprise a modified surface.

181. (New) The method of claim 179, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

182. (New) The method of claim 179, wherein said nanoparticles comprise gold and/or silver.

183. (New) The method of claim 179, wherein each nucleotide is attached to a single nanoparticle or a nanoparticle aggregate.

184. (New) The method of claim 179, further comprising exciting said nucleotides with a laser.

185. (New) The method of claim 184, wherein a charge coupled device (CCD) camera is used to identify said nucleotides.

186. (New) The method of claim 179, further comprising recording the identity of each nucleotide and the time at which each nucleotide is identified.

187. (New) The method of claim 179, wherein said nanoparticles are between 10 nm and 20 micrometers in diameter.

188. (New) A method comprising:

a) removing one or more nucleotides from a nucleic acid;

b) identifying each of the one or more nucleotides by Raman spectroscopy; and

c) determining the sequence of the nucleic acid.

189. (New) The method of claim 188, wherein each type of nucleotide is labeled with a Raman label.

190. (New) The method of claim 188, comprising labeling thymine.

191. (New) The method of claim 188, comprising labeling adenine.

192. (New) The method of claim 188, comprising labeling cytosine.

193. (New) The method of claim 188, comprising labeling guanine.

194. (New) The method of claim 188, comprising labeling uracil.

195. (New) The method of claim 188, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

(Exhibit IV)

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/723,174	11/26/2003	Katrin Kneipp	M0925.70114US01	5755

7590 06/01/2005

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EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 06/01/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/723,174	Applicant(s) KNEIPP ET AL.
	Examiner Ja-Na Hines	Art Unit 1645

JUL 21 2006 PATENT & TRADEMARK OFFICE IAP90

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 September 2004*.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-195* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-195* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

   A. Claims 1-22 are drawn to a method for determining the presence of at least one analyte comprising providing a plurality of aggregates, classified in class 436, subclass 171.

   B. Claims 23-45 are drawn to a method for determining the presence of at least one analyte comprising a plurality of aggregates adsorbing a plurality of analytes, classified in class 424, subclass 9.32.

   C. Claims 46-63 are drawn to a method for determining the presence of at least one analyte comprising a plurality of metal particles, classified in class 436, subclass 73.

   D. Claims 64-77 are drawn to a method for determining the presence of at least one analyte comprising at least one aggregate adsorbing only one analyte, classified in class 436, subclass 173.

   E. Claims 78-90 are drawn to a method for determining the presence of at least one analyte comprising a plurality of surfaces, a portion of the plurality of surfaces adsorbing only one analyte, classified in class 424, subclass 9.351.

   F. Claims 91-95 are drawn to a method for determining the presence of at least one molecule comprising, classified in class 356, subclass 301.

G. Claims 96-106 are drawn to a method for sequencing at least a portion of DNA or RNA, classified in class 424, subclass 9.3.

H. Claims 107-117 are drawn to a method for general field enhancement, classified in class 436, subclass 171.

I. Claims 118-121 are drawn to a method for selecting a spectral range, classified in class 356, subclass 303.

J. Claim 122 is drawn to a method for determining the presence of an analyte comprising providing a sample comprising a rough metal film including a plurality of protrusions and indentations, classified in class 436, subclass 149.

K. Claims 123-124 are drawn to a system for determining the presence of at least one analyte, classified in class 435, subclass 287.2.

L. Claims 125-137 are drawn to a method comprising sequentially removing nucleotides from one end of at least one nucleic acid, classified in class 435, subclass 442.

M. Claims 138-149 are drawn to a method comprising obtaining nucleotides that are attached to Raman labels, classified in class 359, subclass 327.

N. Claims 150-152 are drawn to an apparatus, classified in class 435, subclass 286.5.

O. Claims 153-161 are drawn to a method comprising moving the nucleotides in a stream packed with nanoparticles, classified in class 424, subclass 458.

P. Claims 162-172 are drawn to a method comprising preparing a nucleic acid comprising labeled nucleotides, classified in class 356, subclass 302.

Q. Claims 173-178 are drawn to an apparatus comprising a reaction site for immobilizing a DNA fragment onto an aggregate, classified in class 359, subclass 327.

R. Claims 179-187 are drawn to a method comprising attaching each of one or more nucleotides to at least one nanoparticle, classified in class 435, subclass 6.

S. Claims 188-195 are drawn to a method comprising identifying each of the one or more nucleotides by Raman spectroscopy, classified in class 435, subclass 446.

2. The inventions are distinct, each from the other because of the following reasons:

(I) Inventions A-J, L-M, O-P and R-S are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the instant specification does not disclose that these methods would be used together. Each method has a distinct mode of operation and different effects. For instance, the method of group I is the only method that selects a spectral range, whereas the other methods recite having a different mode of operation along with different functions and effects. Each group has a different function which is unlike the functions of the other groups. In this case, group S identifies each of the one or more nucleotides by Raman spectroscopy, which is unlike group J, which is drawn to

a method for determining the presence of an analyte comprising providing a sample comprising a rough metal film including a plurality of protrusions and indentations. Therefore, the groups have different and unrelated functions. Therefore, each method is divergent in its mode of operation as evidenced by the methods having different functions and effects. For these reasons the inventions of groups A-J, L-M, O-P and R-S are patently distinct.

Furthermore, the distinct methods require separate and distinct searches. The inventions of Groups A-J, L-M, O-P and R-S have acquired a separate status in the art as shown by their different classifications. Moreover, in the instant case, the search for the each method is not coextensive. Group I requires a search drawn to determining the presence of at least one analyte comprising providing a plurality of aggregates, which is not required for the search of the other groups. The prior art teaches that determining the presence of at least one analyte comprising providing a plurality of aggregates, would not necessarily be applicable to a method drawn to obtaining nucleotides that are attached to Raman labels. Likewise, the search for group G which is drawn to a method for sequencing at least a portion of DNA or RNA would require a text search drawn sequencing at least a portion of DNA or RNA, and such a search would not necessarily encompass a search for a method comprising moving the nucleotides in a stream packed with nanoparticles. Therefore, said searches would not necessarily include a search for the other inventions.

Moreover, even if the method for general field enhancement were known, the

method for determining the presence of at least one analyte comprising a plurality of surfaces, a portion of the plurality of surfaces adsorbing only one analyte may be novel and unobvious in view of the preamble. As such, it would be burdensome to search the inventions of groups A-J, L-M, O-P and R-S together.

(II) Inventions K, N and Q are patentably different apparatuses. The apparatuses are distinct as claimed because they have different structures and different uses. Group K is drawn to a system for determining the presence of at least one analyte while Group Q is drawn to an apparatus comprising a reaction site for immobilizing a DNA fragment onto an aggregate. Each group has a different function, effect and is capable of use without the other. For instance, the apparatus of Group Q comprises a reaction site for immobilizing a DNA fragment onto an aggregate as opposed to the Group N which does not. Furthermore, only group K can determine the presence of at least one analyte. Each group has a different structure, produces different effects and has a different function from the other group. Therefore, the apparatuses of the inventions are distinct as claimed.

Furthermore, searching the inventions of groups K,N and Q would impose a serious search burden. The inventions have a separate status in the art as shown by their distinct structure. Thus different apparatuses require different searches. A search for an apparatus comprising a reaction site for immobilizing a DNA fragment onto an aggregate is not necessary for a determination of novelty and unobviousness of the other apparatuses. Moreover, a search of group K is not required to for the apparatus of

group N. Furthermore, the apparatus of group Q may be known even if the apparatus of group K is novel. In addition, the technical literature search for the apparatus of group N and the apparatus of group K are not coextensive, e.g., the apparatus of group N may be characterized in the technical literature prior to discovery of group K.

3. Because these inventions are distinct for the reasons given above, and have acquired a separate status in the art as shown by their different classification, the search required for each group is not required for the other groups since each group requires a different non-patent literature search due to each group comprising different method steps, restriction for examination purposes as indicated is proper.

4. Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ja-Na Hines whose telephone number is 571-272-0859. The examiner can normally be reached on Monday-Thursday and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith can be reached on 571-272-0864. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Ja-Na Hines
May 24, 2005

AUG 29 2005 PATENT & TRADEMARK OFFICE

JUL 21 2006 PATENT & TRADEMARK OFFICE

(Exhibit V)

DOCKET NO.: M0925.70114US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Kneipp, et al.
Serial No.:	10/723,174
Confirmation No.:	5755
Filed:	November 26, 2003
For:	SINGLE MOLECULE DETECTION WITH SURFACE-ENHANCED RAMAN SCATTERING AND APPLICATIONS IN DNA OR RNA SEQUENCING
Examiner:	Ja-Na Hines
Art Unit:	1645

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to MAIL STOP AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 25th day of August, 2005.

Jeannie McAlexander
Signature

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to examination, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims that begins on page 2 of this amendment.

Remarks begin on page 22 of this amendment.

In the Claims

Applicants present replacement claims below indicating the changes with insertions indicated by underlining and deletions indicated by strikeouts.

1. (Currently Amended) ~~A~~ The method ~~for determining the presence of at least one analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a plurality of aggregates of size of at least about 500 nm;

adsorbing ~~a plurality of analytes~~ at least some of the one or more nucleotides to at least some of the plurality of aggregates;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein at least one spectral line of the information represents a single ~~analyte~~ nucleotide adsorbed on one of the plurality of aggregates; and

determining the presence of the single ~~analyte~~ nucleotide from the at least one spectral line.

2. (Currently Amended) A method as in claim 1, the exposing step ~~involving~~ comprising exposing the sample to electromagnetic radiation and causing Raman scattering of the sample, and the obtaining step comprising obtaining Raman information of the sample, wherein a single Raman line of the information represents the single ~~analyte~~ nucleotide.

3. (Original) A method as in claim 1, wherein the sample is free of an emission-enhancing aid.

4. (Original) A method as in claim 1, wherein the spectral information is a surface-enhanced Raman spectrum, having an enhancement factor of at least about 10^{10}.

5. (Original) A method as in claim 1, wherein each aggregate of the plurality of aggregates comprises a plurality of metal particles.

6. (Currently Amended) A method as in claim 5, wherein ~~the plurality~~ at least some of the metal particles ~~is~~ are selected from the group consisting of silver, gold and copper particles.

7. (Currently Amended) A method as in claim 6, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

8. (Original) A method as in claim 1, wherein the plurality of aggregates is selected from the group consisting of colloids suspended in a medium, aggregates deposited on a substrate and lithography-produced metal aggregates.

9. (Original) A method as in claim 8, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

10. (Original) A method as in claim 8, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

11. (Original) A method as in claim 1, wherein the sample consists essentially of a plurality of aggregates of from about 500 nm to about 20 microns in dimension.

12. (Original) A method as in claim 1, wherein the electromagnetic radiation is non-resonant radiation.

13. (Original) A method as in claim 12, wherein the electromagnetic radiation is near infrared radiation.

14. (Original) A method as in claim 1, wherein the spectral information is Raman information that defines less than a complete Raman spectrum.

15. (Original) A method as in claim 14, wherein the spectral information is less than 5 Raman lines.

16. (Original) A method as in claim 14, wherein the spectral information is less than 2 Raman lines.

17. (Original) A method as in claim 1, wherein the spectral information is a single Raman line.

18. (Cancelled)

19. (Currently Amended) A method as in claim 1, wherein the single ~~analyte~~ nucleotide is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

20-22. (Cancelled)

23. (Currently Amended) ~~A~~ The method ~~for determining the presence of an analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a plurality of aggregates;

adsorbing ~~a plurality of analytes~~ each of the one or more nucleotides to at least some of the plurality of aggregates, wherein at least one aggregate of the plurality of aggregates comprises a metal cluster of at least seven particles and adsorbs only one ~~analyte~~ nucleotide;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein the only one ~~analyte~~

nucleotide contributes to the spectral information; and

determining the presence of the only one ~~analyte~~ nucleotide from the spectral information.

24. (Currently Amended) A method as in claim 23, the exposing step ~~involving~~ comprising exposing the sample to electromagnetic radiation to cause Raman scattering, and the obtaining step ~~involves~~ comprises obtaining a Raman spectrum of the sample, wherein the only one ~~analyte~~ nucleotide contributes to at least one Raman signal of the Raman spectrum.

25. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least ten particles.

26. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least twenty particles.

27. (Original) A method as in claim 23, wherein the plurality of aggregates comprises a metal cluster of at least thirty-five particles.

28. (Original) A method as in claim 23, wherein the sample is free of an emission-enhancing aid.

29. (Currently Amended) A method as in claim ~~23~~ 24, wherein the Raman spectrum is a surface-enhanced Raman spectrum, having an enhancement factor of at least ~~1010~~ 10^{10}.

30. (Currently Amended) A method as in claim 23, wherein the metal cluster of at least seven particles ~~are~~ comprises particles selected from the group consisting of silver, gold and copper particles.

31. (Currently Amended) A method as in claim 23, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

32. (Original) A method as in claim 23, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

33. (Original) A method as in claim 32, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

34. (Original) A method as in claim 32, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

35. (Currently Amended) A method as in claim 23, wherein at least some of the ~~at least one~~ aggregates ~~has~~ have a dimension of at least about 500 nm.

36. (Original) A method as in claim 23, wherein the electromagnetic radiation is non-resonant radiation.

37. (Original) A method as in claim 36, wherein the electromagnetic radiation is near infrared radiation.

38. (Cancelled)

39. (Currently Amended) A method as in claim 23, wherein the ~~single analyte~~ only one nucleotide is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

40-42. (Cancelled)

43. (Original) A method as in claim 23, wherein the sample consists essentially of aggregates of size of from about 500 nm to about 20 microns.

44. (Currently Amended) A method as in claim 23, wherein the ~~at least one~~ plurality of aggregates comprises a plurality of metal particles each having a dimension of no more than about 100 nm.

45. (Currently Amended) A method as in claim 23, wherein the ~~at least one~~ plurality of aggregates comprises a plurality of metal particles each having a dimension of no more than about 75 nm.

46. (Currently Amended) ~~A~~ The method ~~for determining the presence of an analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a plurality of aggregates;

adsorbing ~~a plurality of analytes~~ at least some of the one or more nucleotides to at least some of the plurality of aggregates, wherein each aggregate comprises a plurality of metal particles, each metal particle having a dimension of no more than about 100 nm and at least one aggregate adsorbs only one ~~analyte~~ nucleotide;

exposing the sample to electromagnetic radiation to cause surface-enhanced emission;

obtaining spectral information of the sample, wherein the only one ~~analyte~~ nucleotide contributes to the spectral information; and

determining the presence of the only one ~~analyte~~ nucleotide from the spectral information.

47. (Currently Amended) A method as in claim 46, wherein the exposing step ~~involves~~ comprises causing surface-enhanced emission and the obtaining step ~~involves~~ comprises obtaining Raman spectral information.

48. (Original) A method as in claim 46, wherein the sample is free of an emission-enhancing aid.

49. (Currently Amended) A method as in claim 46, wherein the spectral information is a surface-enhanced Raman spectrum, having an enhancement factor of at least ~~1010~~ 10^{10}.

50. (Currently Amended) A method as in claim 46, wherein at least some of the metal particles are selected from the group consisting of silver, gold and copper particles.

51. (Currently Amended) A method as in claim 46, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

52. (Original) A method as in claim 46, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

53. (Original) A method as in claim 52, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

54. (Original) A method as in claim 52, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

55. (Original) A method as in claim 46, each metal particle having a dimension of no more than about 75 nm.

56. (Original) A method as in claim 46, wherein the electromagnetic radiation is non-resonant radiation.

57. (Original) A method as in claim 56, wherein the electromagnetic radiation is near infrared radiation.

58. (Original) A method as in claim 46, wherein the spectral information consists essentially of less than 5 lines of a Raman spectrum.

59. (Cancelled)

60. (Currently Amended) A method as in claim 46, wherein the ~~single analyte~~ only one nucleotide is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

61-63. (Cancelled)

64. (Currently Amended) ~~A~~ The method ~~for determining the presence of at least one analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a plurality of aggregates~~,~~;

to at least one aggregate, adsorbing only one ~~analyte~~ nucleotide that is free of an emission-enhancing aid;

exposing the sample to electromagnetic radiation; and

obtaining a spectrum, wherein the only one ~~analyte~~ nucleotide contributes to at least one signal of the spectrum.

65. (Currently Amended) A method as in claim 64, wherein the spectrum is a surface-enhanced Raman spectrum, having an enhancement factor of at least ~~1010~~ 10^{10}.

66. (Original) A method as in claim 64, wherein each aggregate of the plurality of aggregates comprises a plurality of metal particles.

67. (Currently Amended) A method as in claim 66, wherein at least some of the metal particles are selected from the group consisting of silver, gold and copper particles.

68. (Original) A method as in claim 64, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

69. (Original) A method as in claim 64, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

70. (Original) A method as in claim 69, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

71. (Original) A method as in claim 69, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

72. (Currently Amended) A method as in claim 64, wherein at least some of the ~~at least one~~ aggregates ~~has~~ have a dimension of at least about 500 nm.

73. (Cancelled)

74. (Currently Amended) A method as in claim 64, wherein the ~~single analyte~~ only one nucleotide is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

75-77. (Cancelled)

78. (Currently Amended) ~~A~~ The method ~~for determining the presence of a single analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a plurality of surfaces;

to a portion of the plurality of surfaces, adsorbing only one ~~analyte~~ nucleotide;

and

exposing the sample to electromagnetic radiation to cause the sample to emit radiation such that the sample is free of photobleaching.

79. (Original) A method as in claim 78, wherein the plurality of surfaces comprises a plurality of aggregates.

80. (Original) A method as in claim 79, wherein the plurality of aggregates comprises a plurality of metal particles.

81. (Currently Amended) A method as in claim 80, wherein at least some of the metal particles are selected from the group consisting of silver, gold and copper particles.

82. (Original) A method as in claim 79, wherein the plurality of aggregates is selected from the group consisting of a colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

83. (Original) A method as in claim 82, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

84. (Original) A method as in claim 82, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

85. (Original) A method as in claim 78, wherein the plurality of surfaces comprises a plurality of aggregates of metal particles, each of the metal particles having a dimension of no more than about 100 nm.

86. (Cancelled)

87. (Currently Amended) A method as in claim 78, wherein the only one ~~analyte~~ nucleotide is selected from the group consisting of thymine, adenine, cytosine, guanine, and uracil.

88-90. (Cancelled)

91. (Currently Amended) ~~A~~ The method ~~for determining the presence of at least one molecule, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises ~~providing at least one molecule,~~ exposing ~~the at least one molecule~~ each of the one or more nucleotides to electromagnetic radiation to cause Raman scattering, obtaining Raman spectral information, and determining the presence of ~~the at least one molecule~~ each of the one or more nucleotides from at least one anti-Stokes line.

92. (Currently Amended) A method as in claim 91, wherein ~~the at least one molecule~~ each of the one or more nucleotides is adsorbed on a plurality of surfaces.

93. (Currently Amended) A method as in claim 91, wherein ~~the~~ at least one ~~analyte~~ nucleotide is exposed to non-resonant radiation.

94. (Currently Amended) A method as in claim ~~92~~ 91, wherein the electromagnetic radiation is near infrared radiation.

95. (Original) A method as in claim 94, wherein the near infrared radiation has a wavelength of at least 1000 nm.

96. (Currently Amended) ~~A~~ The method ~~for sequencing at least a portion of DNA or RNA, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

~~cleaving the at least a portion of DNA or RNA into DNA or RNA fragments, wherein each fragment comprises at least one base;~~

allowing each ~~DNA or RNA fragment~~ of the one or more nucleotides to become surface-adsorbed;

exposing each ~~fragment~~ of the one or more nucleotides to electromagnetic radiation to cause surface-enhanced emission; and

obtaining unique surface-enhanced spectral information attributed to each ~~fragment~~ of the one or more nucleotides.

97. (Currently Amended) A method as in claim 96, wherein each ~~fragment~~ of the one or more nucleotides is surface-adsorbed onto one of a plurality of surfaces.

98. (Original) A method as in claim 97, wherein the plurality of surfaces is included in a moving stream.

99. (Currently Amended) A method as in claim 97, wherein the ~~plurality of~~ surfaces ~~is~~ are surfaces of aggregates, the aggregates being selected from the group consisting of a plurality of aggregates suspended in a medium, a plurality of aggregates deposited on a substrate and lithography produced metal aggregates.

100. (Original) A method as in claim 99, wherein the plurality of aggregates comprise clusters of metal particles.

101. (Currently Amended) A method as in claim 100, wherein at least some of the metal particles are selected from the group consisting of silver, gold and copper particles.

102. (Original) A method as in claim 99, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

103. (Original) A method as in claim 100, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

104. (Original) A method as in claim 96, comprising allowing each ~~fragment~~ of the one or more nucleotides to become surface-absorbed on a plurality of protrusions and voids on a rough metal film.

105. (Original) A method as in claim 96, wherein the electromagnetic radiation is non-resonant radiation.

106. (Original) A method as in claim 96, wherein the electromagnetic radiation is near infrared radiation.

107. (Currently Amended) ~~A~~ The method ~~for general field enhancement, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises providing a plurality of aggregates, attaching each of the one or more nucleotides to one or more aggregates, exposing the plurality of aggregates to near infrared radiation, and inducing at least one electromagnetic resonance in the plurality of aggregates to cause a surface-enhanced radiation.

108. (Original) A method as in claim 107, wherein the near infrared radiation has a wavelength of at least 1000 nm.

109. (Original) A method as in claim 107, wherein the plurality of aggregates comprises a plurality of metal particles.

110. (Original) A method as in claim 109, wherein at least some of the metal particles are selected from the group consisting of silver, gold and copper particles.

111. (Currently Amended) A method as in claim 107, wherein the plurality of aggregates is formed in situ by exposure to the electromagnetic radiation.

112. (Original) A method as in claim 107, wherein the plurality of aggregates is selected from the group consisting of colloids suspended in a medium, aggregates deposited on a substrate and lithography produced metal aggregates.

113. (Original) A method as in claim 112, wherein the medium is selected from the group consisting of water, an organic solvent and a gel.

114. (Original) A method as in claim 112, wherein the substrate is selected from the group consisting of an electrode, a glass layer and a quartz layer.

115. (Original) A method as in claim 109, wherein each metal particle has a dimension of no more than about 100 nm.

116. (Original) A method as in claim 109, wherein the plurality of aggregates comprises at least seven metal particles.

117. (Currently Amended) A method as in claim 107, wherein the surface enhanced radiation has an enhancement factor of at least ~~1010~~ 10^{10}.

118-121. (Cancelled)

122. (Currently Amended) ~~A~~ The method ~~for determining the presence of an analyte, comprising~~ of claim 188, wherein the act of identifying each of the one or more nucleotides comprises:

providing a sample comprising a rough metal film including a plurality of protrusions and indentations;

absorbing ~~a plurality of analytes~~ the one or more nucleotides on a surface of the film;

exposing the sample to electromagnetic radiation to cause Raman scattering; and

obtaining a unique Raman signal attributed to a single ~~analyte~~ nucleotide.

123-124. (Cancelled)

125. (Currently Amended) ~~A~~ The method of claim 188, comprising:

~~a)~~ sequentially removing the one or more nucleotides from one end of ~~at least one~~ the nucleic acid~~;~~

~~b) attaching each nucleotide to at least one nanoparticle;~~

~~c) identifying said nucleotides; and~~

~~d) determining the sequence of said nucleic acid~~.

126. (Currently Amended) The method of claim ~~125~~ 188, wherein said nucleic acid is attached to a surface.

127. (Cancelled)

128. (Currently Amended) The method of claim ~~125~~ 188, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

129. (Cancelled)

130. (Currently Amended) The method of claim ~~129~~ 188, wherein each nucleotide is attached to a single nanoparticle or a nanoparticle aggregate.

131. (Cancelled)

132. (Previously Presented) The method of claim 128, further comprising exciting said nucleotides with a laser.

133. (Previously Presented) The method of claim 132, wherein a charge coupled device (CCD) camera is used to identify said nucleotides.

134. (Currently Amended) The method of claim ~~125~~ 188, further comprising recording the identity of each nucleotide and the time at which each nucleotide is identified.

135. (Currently Amended) The method of claim ~~125~~ 188, wherein an exonuclease is used to remove said nucleotides from said nucleic acid.

136-137. (Cancelled)

138. (Currently Amended) ~~A~~ The method of claim 188, further comprising, prior to the act of removing one or more nucleotides from the nucleic acid:

~~a)~~ obtaining one or more nucleotides that are attached to Raman labels; and

~~b)~~ providing a nucleic acid comprising the labeled nucleotides~~;~~

~~c) removing nucleotides from one end of the nucleic acid;~~

~~d) identifying nucleotides by Raman spectroscopy; and~~

~~e) determining the sequence of the nucleic acid~~.

139. (Currently Amended) The method of claim ~~138~~ 188, further comprising passing the nucleotides removed from the nucleic acid in a stream.

140-145. (Cancelled)

146. (Currently Amended) The method of claim ~~138~~ 188, wherein said nucleotides are removed from said nucleic acid by exonuclease activity.

147. (Previously Presented) The method of claim 146, wherein only one nucleic acid at a time is exposed to exonuclease activity.

148-152. (Cancelled)

153. (Currently Amended) ~~A~~ The method of claim 188, comprising:

~~a) sequentially removing nucleotides from one end of at least one nucleic acid;~~

~~b)~~ moving the nucleotides in a stream packed with nanoparticles~~;~~

~~c) identifying the nucleotides by Raman spectroscopy; and~~

~~d) determining the sequence of the nucleic acid~~.

154. (Cancelled)

155. (Currently Amended) The method of claim ~~153~~ 188, further comprising attaching said nucleic acid to a surface.

156. (Currently Amended) The method of claim ~~155~~ 188, wherein said nucleic acid is immobilized in a reaction site.

157. (Previously Presented) The method of claim 156, wherein a single nucleic acid is immobilized in said reaction site.

158. (Cancelled)

159. (Previously Presented) The method of claim 153, wherein at least two nanoparticles are cross-linked together.

160. (Previously Presented) The method of claim 153, wherein the nanoparticles comprise gold and/or silver, said nanoparticles between about 10 nm and 20 micrometers in size.

161. (Previously Presented) The method of claim 160, wherein the size of said nanoparticles is selected from the group consisting of about 10 to 50 nm, about 10 to 100 nm, about 10 nm and about 500 nm.

162. (Currently Amended) ~~A~~ The method of claim 188, further comprising:

~~a)~~ preparing a nucleic acid comprising labeled nucleotides~~;~~

~~b) sequentially removing nucleotides from one end of the nucleic acid;~~

~~c) moving the nucleotides in a stream packed with nanoparticles;~~

~~d) identifying the nucleotides by Raman spectroscopy; and~~

~~e) determining the sequence of the nucleic acid~~.

163. (Cancelled)

164. (Previously Presented) The method of claim 189, wherein each type of nucleotide is labeled with a distinguishable Raman label.

165-171. (Cancelled)

172. (Currently Amended) The method of claim ~~13~~ 188, wherein each type of nucleotide produces a unique Raman signal.

173-178 (Cancelled)

179. (Currently Amended) ~~A~~ The method of claim 188, further comprising:

~~a) removing one or more nucleotides from a nucleic acid;~~

~~b)~~ attaching each of the one or more nucleotides to at least one nanoparticle~~;~~

~~c) identifying said nucleotides; and~~

~~d) determining the sequence of said nucleic acid~~.

180. (Currently Amended) The method of claim 179, wherein said at least one nanoparticles comprises a modified surface.

181. (Cancelled)

182. (Previously Presented) The method of claim 179, wherein said nanoparticles comprise gold and/or silver.

183. (Previously Presented) The method of claim 179, wherein each nucleotide is attached to a single nanoparticle or a nanoparticle aggregate.

184-186. (Cancelled)

187. (Previously Presented) The method of claim 179, wherein said nanoparticles are between 10 nm and 20 micrometers in diameter.

188. (Previously Presented) A method comprising:

a) removing one or more nucleotides from a nucleic acid;

b) identifying each of the one or more nucleotides by Raman spectroscopy; and

c) determining the sequence of the nucleic acid.

189. (Previously Presented) The method of claim 188, wherein each type of nucleotide is labeled with a Raman label.

190. (Currently Amended) The method of claim 188, ~~comprising labeling~~ wherein the nucleic acid comprises labeled thymine.

191. (Currently Amended) The method of claim 188, ~~comprising labeling~~ wherein the nucleic acid comprises labeled adenine.

192. (Currently Amended) The method of claim 188, ~~comprising labeling~~ wherein the nucleic acid comprises labeled cytosine.

193. (Currently Amended) The method of claim 188, ~~comprising labeling~~ wherein the nucleic acid comprises labeled guanine.

194. (Currently Amended) The method of claim 188, ~~comprising labeling~~ wherein the nucleic acid comprises labeled uracil.

195. (Previously Presented) The method of claim 188, wherein said nucleotides are identified by surface enhanced Raman spectroscopy (SERS) and/or surface enhanced resonance Raman spectroscopy (SERRS).

196. (New) The method of claim 179, wherein the act of attaching each of the one or more nucleotides to at least one nanoparticle occurs prior to the act of removing one or more nucleotides from a nucleic acid.

197. (New) The method of claim 179, wherein the act of attaching each of the one or more nucleotides to at least one nanoparticle occurs after the act of removing one or more nucleotides from a nucleic acid.

198. (New) The method of claim 188, wherein the one or more nucleotides is free of an emission-enhancing aid.